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Characterization of Spotted Fever Group Rickettsiae in Flea and Tick Specimens from Northern Peru

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Evidence of spotted fever group (SFG) rickettsiae was obtained from flea pools and individual ticks collected at three sites in northwestern Peru within the focus of an outbreak of febrile disease in humans attributed, in part, to SFG rickettsia infections. Molecular identification of the etiologic agents from these samples was determined after partial sequencing of the 17-kDa common antigen gene (*htrA*) as well as pairwise nucleotide sequence homology with one or more of the following genes: *gltA*, *ompA*, and *ompB*. Amplification and sequencing of portions of the *htrA* and *ompA* genes in pooled samples (2 of 59) taken from fleas identified the pathogen *Rickettsia felis*. Four tick samples yielded molecular evidence of SFG rickettsiae. Fragments of the *ompA* (540-bp) and *ompB* (2,484-bp) genes were amplified from a single *Amblyomma maculatum* tick (tick 124) and an *Ixodes boliviensis* tick (tick 163). The phylogenetic relationships between the rickettsiae in these samples and other rickettsiae were determined after comparison of their *ompB* sequences by the neighbor-joining method. The dendrograms generated showed that the isolates exhibited close homology (97%) to *R. aeschlimannii* and *R. rhipicephali*. Significant bootstrap values supported clustering adjacent to this nodule of the SFG rickettsiae. While the agents identified in the flea and tick samples have not been linked to human cases in the area, these results demonstrate for the first time that at least two SFG rickettsia agents were circulating in northern Peru at the time of the outbreak. Furthermore, molecular analysis of sequences derived from the two separate species of hard ticks identified a possibly novel member of the SFG rickettsiae.

Proteobacteria of the family *Rickettsiaceae*, order *Rickettsiales*, are made up of highly specialized obligate intracellular, gram-negative bacteria that survive freely within the cytosol of the host cell. Members of the genus *Rickettsia* are divided into two genetically similar groups, the spotted fever group (SFG) and the typhus group (TG), on the basis of host specificity, intracellular location, in vitro growth conditions, antigenic characteristics, the molecular sequences of conserved genes, clinical features, and epidemiology (15, 16, 37, 38). Seventeen species of the genus *Rickettsia* are categorized within the SFG rickettsiae. With the exception of *Rickettsia akari* (mite-borne) and *R. felis* (flea-borne), the remaining SFG rickettsia species are recognized as tick-borne rickettsiae that are passed to subsequent generations or stages transovarially and transtadially (21). While the members of the SFG rickettsiae are adapted to existence within specific hosts, they are capable of infecting humans after humans are bitten by infected arthropods. The TG contains two species, *R. prowazekii* and *R. typhi*. The former scrub typhus group has been moved into its own genus, *Orientia tsutsugamushi*, following analysis of the 16S rRNA gene (47).

At least 15 members of SFG rickettsiae have been associated with syndromic diseases that result in diverse clinical presentations, from asymptomatic to severe (25, 49). The illnesses that occur as a result of rickettsial infections are often characterized by an acute onset of fever, accompanied by nonspecific signs and symptoms. Occasionally, a rash follows the occurrence of the systemic symptoms and may be pathognomonic (50). Clinical presentation and manifestations often vary, with the more pathogenic strains causing debilitating disease with rapid onset.

While rickettsioses have been reported on practically every continent, the incidence of rickettsial diseases has been underreported in Central and South America (20) and has generally been undocumented in Peru. Brazilian spotted fever, which is transmitted by the tick *Amblyomma cajennense* (12), was initially reported in Brazil more than 60 years ago (14), and cases have increasingly been documented over the last 20 years (10, 11, 13, 43, 46). Rickettsiae and rickettsioses have also been recorded in Mexico (52), Costa Rica (19), and Argentina (27, 39). The SFG rickettsia agent, *R. felis*, has been identified in domestic animals and is thought to infect humans in Peru, although these data remain unpublished (C. Moron, unpublished data). Louse-transmitted typhus infections have previously plagued sierra communities in the department of Cusco in central Peru (33).

Between May and October 2002, a number of febrile cases, including two deaths, were reported in the area around the town of Sapillica in northwestern Peru. A joint investigation

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TABLE 1. Description of primers used to amplify gene fragments

Target gene or species	Primer or probe	Sequence (5′–3′)[a]	Amplicon size (bp)
htrA	R17-122 (first round)	CAGAGTGCTATGAACAAACAAGG	
	R17-500 (first round)	CTTGCCATTGCCCATCAGGTT	378
	TZ15 SFG F	TTCTCAATTCGGTAAGGGC	
	TZ16 SFG R	ATATTGACCAGTCGCTATTTC	247
	RPID TG F	CGGTACACTTCTTGGTGGCGCAGGAGGT	
	RP2 TG R	TTCACGGCAATATTGACCTGTACTGTTCC	286
htrA 434-bp fragment	Rr1175F	GCTCTTGCAACTTCTATGTT	
	Rr2608R	CATTGTTCGTCAGGTTGGCG	434
ompB[c] qPCR	RF1396F	ACCCAGAACTCGAACTTTGGTG	
	RF1524R	CACACCCGCAGTATTACCGTT	
R. felis[b]	RFQuBP (probe)	CGCGACTTACAGTTCCTGATACTAAGGTTCTT ACAGGTCGCG-BHQ-2	129
ompB qPCR	RR1595F	GCCGGAGTTGTCCAATTATCA CCGCCGACAAGAGCAGTTT	128
SFG; not *R. felis* or *R. akari*	RR1722R	ROX-CCGCGCCGGCATTTCCTAAACGTAACTCGGC	
	RR1654P	AGCGCGG-DAB	
ompB	ompB2409F	CCGTAACATTAAACAAACAAGCTG	2,478
	ompB4887R	AGAGTACCTTGATGTGCRGTATAYT	
ompA	RR 190-70 (first round)	ATGGCGAATATTTCTCCAAAA	
	RR 190-701 (first round)	GTTCCGTTAATGGCAGCATCT	590
	190-FN1 (nested)	AAGCAATACAACAAGGTC	
	190-RN1 (nested)	TGACAGTTATTATACCTC	540
gltA	RpCS.877p	GGGGGCCTGCTCACGGCGG	
	RpCS. 1258n	ATTGCAAAAAGTACAGTGAACA	381

[a] Abbreviations: ROX, passive reference dye; DAB, diaminobenzidine; Y, pyrimidine.
[b] Primers and probe specific for amplication of *ompB* gene fragment of *R. felis* (J. Jiang, unpublished).
[c] Primers specific for amplication of *ompB* gene fragment of SFG and not *R. felis* (J. Jiang, unpublished).

with representatives of the Peruvian Ministry of Health documented a high seroprevalence of rickettsial agents and *Leptospira* species and the molecular identification of an SFG rickettsia (7). As these diseases are often associated with a zoonotic focus, ectoparasites were taken from domestic animals and animals trapped in the wild during the course of this study. Here we provide evidence from the results of PCR, sequencing, and phylogenetic analyses of fragments of the common 17-kDa antigen gene (*htrA*) (2), the citrate synthase gene (*gltA*) (24), *ompA* (18), and *ompB* (24, 41) that SFG rickettsiae were circulating among arthropods at the time of the disease outbreak. Finally, the 2,484-bp sequence of the *ompB* gene obtained from two ticks was found to be phylogenetically disparate (≥3%) from those of known SFG rickettsia species, suggesting a putative novel species.

MATERIALS AND METHODS

Ecologic and entomologic samples. Ecologic and entomologic samples were obtained from the sparsely populated sierras of northwestern Peru in an area that rests roughly 2,700 m above sea level, has a mean average temperature range from −3 to 34°C, and rainfall of 1,000 mm/year. Within the defined study area, which comprised three separate villages 45 km apart, rodents were trapped live by using a combination of Tomahawk (Tomahawk Live Trap Co., Tomahawk, Wis.) and Sherman (H. B. Sherman Traps Inc., Tallahassee, Fla.) traps. Additionally, domesticated dogs, cats, and horses were gathered and screened for fleas and ticks. Ectoparasites were removed with fine forceps and placed individually in vials containing 70% ethanol or snap frozen in liquid nitrogen. DNA was prepared from these as described previously (44).

PCR. Nested PCR for the detection of fragments of the *Rickettsia* genus-specific 17-kDa protein gene (*htrA*) was performed to discern *R. typhus* and *R. rickettsii* members by using the sequences published previously (48). Characterization of SFG rickettsiae was determined after single-stage or nested PCR of specific conserved genes, including *ompA* (40) and the citrate synthase (*gltA*) genes (42). The PCR primers and the sizes of the amplicons obtained following amplification are shown in Table 1. Reactions were performed with Ready-To-Go PCR Beads (Amersham Pharmacia Biotech) with *Taq* Platinum (Invitrogen, Inc., Carlsbad, Calif.). Primers were used at 0.5 to 1.0 μmol/liter, with $MgCl_2$ concentrations held at 1.5 mmol/liter. Following electrophoresis on ethidium bromide-stained 2% low-melting-temperature agarose gels, the PCR products were purified and sequenced on either an automated ABI 373A or an ABI Prism 3100 gene sequencer (Applied Biosystems, Foster City, Calif.) by standard procedures.

For the extended *ompB* amplifications (41), PCR primers with specificities for conserved regions were designed after alignment of the sequences of 23 SFG rickettsiae (J. Jiang, unpublished data). A nested PCR was performed with one reverse primer and two different forward primers (Table 1). One microliter of the DNA template was added to the reaction mixture, which contained SuperMix High Fidelity (Invitrogen) and 0.5 μM primers. Each PCR was conducted in a T-Gradient thermocycler (Biometra, Goettingen, Germany). Following initial denaturation for 1 min at 94°C, 35 cycles of denaturation for 30 s at 94°C, annealing for 1 min at 50°C, and extension for 4 min at 68°C were performed. A final extension step was done for 20 min at 72°C. The PCR products were purified and cloned with a TOPO XL PCR cloning kit (Invitrogen), following the instructions of the manufacturer. Plasmid DNA was isolated (Miniprep; Qiagen) from one colony each for tick 124 and tick 163. The sequence was obtained with both forward and reverse sequencing primers, as indicated below, with an ABI Prism 3100 genetic analyzer (Applied Biosystems). The sequences were assembled with Sequencher (version 4.0) (Gene Codes Corporation Inc., Ann Arbor, Mich.).

Sequencing primers. The following M13 forward and reverse primers from the Topo XL PCR cloning kit (Invitrogen) were used: primer ∗120-2788F (AAAC AATAATCAAGGTACTGT), primer R3521F (GATAATGCCAATGCAAAT TTCAG), primer R4224F (ACCAAGATTATAAGAAAGGTGATAA), primer R3008R (CGCCTGTAGTAACAGTTACAC), primer R3637R (GAAACGAT TACTTCCGGTTACA), and primer ∗120-4346R (CGAAGAAGTAACGCTG ACTT).

qPCR. A *Rickettsia* genus-specific quantitative real-time PCR (qPCR) (26) and an *R. felis*-specific qPCR were performed to detect the presence of the *R. felis ompB* gene. The *R. felis* qPCR assay was specifically designed to detect a portion of the *R. felis ompB* gene (in the open reading frame between positions 1179 and 1307). Preparations of DNA from *R. felis*-infected cat fleas (*Ctenocephalides felis*; FleaData, Inc. Freeville, N.Y.) (8) were positive by this assay; but those of DNA from *R. typhi* Wilmington or Museibov strains, *R. prowazekii*, 11 species of SFG rickettsiae, *Orientia*, *Ehrlichia*, *Bartonella*, and 12 other bacteria were not (J. Jiang, unpublished). The host cell DNA included in the bacterial cultures did not produce false-positive reactions. For the *R. felis* qPCRs, 2 μl of template was reacted with 0.5 μM both forward and reverse primers and 0.4 μM Quasar 670 probe (Biosearch Technologies, Inc., Novato, Calif.). $MgCl_2$ (5 mM) and premixed OmniMix HS beads (Cepheid, Sunnyvale, Calif.) were added to the reaction mixture. Amplification for the qPCR assay was conducted with a SmartCycler thermocycler (Cepheid) with the following temperatures and cycle parameters: an initial denaturation of 3 min at 94°C and then 50 cycles of denaturation (94°C for 5 s) and annealing and elongation (60°C for 30 s). The threshold cycle value was determined when experimental samples produced fluorescence greater than the calculated threshold cycle value on the basis of the background fluorescence measured during amplification. No-template controls (which contained 2 μl of double-distilled H_2O instead of DNA), which were produced at the same time and under the same conditions as the experimental positive control samples, were consistently negative (i.e., the fluorescence did not cross the threshold of background fluorescence).

Phylogenetic analysis. The sequences were compared to those downloaded from GenBank by using MacVector (version 7.22) software (Accelrys, Inc., San Diego, Calif.), and alignments were created with Sequencher (version 3.0) software (Gene Codes Corporation Inc.). Phylogenetic analyses were performed with the PAUP (version 5.0) program (Sinauer Associates, Inc., Sunderland, Mass.) with the criterion of maximum parsimony and neighbor joining. Reference sequences from GenBank and two voucher specimens (*R. akari* and *R. rickettsii*) were used to confirm the results for the experimental samples. For the maximum-parsimony analysis, all characters were weighted equally, and a heuristic search with 100 replicates of a random taxon was performed to facilitate branch swapping. Confidence in nodes was assessed by the bootstrap procedure with 500 resampling replicates. Bootstrap values >70% were considered well supported.

Nucleotide sequence accession numbers. The sequences obtained from the DNA extracted from tick 163 were assigned GenBank accession numbers AY590796 (540 bp, *ompA*), AY590797 (381 bp, *gltA*), and AY652981 (2,484 bp, *ompB*).

RESULTS

Sample collection. Sampling was conducted at three sites during an outbreak of febrile illness that began in June 2002 and that ended in November 2002. These sites, Coletas, Naranjo, and Sapillica, are located in the department of Piura, northeast of the city of Sullana, in northwestern Peru. A total of 16 ticks and 59 pools of fleas were collected and categorized (Table 2). Ectoparasites (fleas and ticks) were taken from individual domestic animals and animals trapped in the wild and sorted by species. Ticks were identified by using taxonomic keys for the tick species found in Peru (28). A total of six tick species and six flea species were collected from dogs, horses, cats, and rodents. No ticks or fleas were taken from human subjects in the area.

PCR and qPCR analysis of *R. felis* from flea samples. Once the fleas were keyed and sorted, DNA was extracted from pools removed from a single domestic animal or an animal trapped in the wild. Initially, all samples were tested by PCR for detection of the 17-kDa common antigen gene (*htrA*) found in both TG and SFG members of the rickettsiae (2, 48). In the initial reaction, genus-specific primers were used to amplify a PCR product, and then a nested reaction was conducted to differentiate the TG from the SFG members of the rickettsiae. Nested reactions for the TG rickettsiae yielded a product of 286 bp, while reactions for the SFG rickettsiae gave a product of 247 bp. The results of the analyses was confirmed by PCR and sequencing of a 434-bp amplicon (51). After screening, 2 of 59 pools of fleas were found to contain PCR bands and sequences that signified an infection with an SFG agent. Both samples were extracted from pools of *C. felis* fleas removed from domestic canines in Sapillica. In flea sample FL105, an SFG-specific band and a TG-specific band were both evident (Fig. 1A and B). In order to rule out possible dual infection, a qPCR assay specific for *R. typhi* (J. Jiang, unpublished) was conducted, and the results were found to be negative (data not shown). Sequence analysis of the *htrA* PCR product demonstrated 100% similarity with that of *R. felis* (GenBank accession number AF195118.1). To differentiate the possible SFG agent in the flea samples, *ompB* qPCR assays specific for either *R. felis* or SFG rickettsiae but not *R. felis* were conducted. Flea samples FL103 and FL105 were positive by the *R. felis* qPCR. The 129-bp *R. felis* PCR amplicon produced by FL105 is shown in Fig. 1. Additional characterization of the nucleic acid preparations from the flea samples was attempted by using a PCR assay specific for a portion of the *ompA* gene. A 540-bp PCR product for *ompA* was obtained from sample FL105 and sequenced. This product also demonstrated 100% homology with the *R. felis* sequence. Collectively, these data confirm the presence of *R. felis* within *C. felis* fleas collected in Peru.

TABLE 2. Ectoparasite collected and percentage of parasites positive for an SFG agent

Ectoparasite	No. (% of total[a])	No. SFG positive (% of total[a])
Tick species		
Amblyomma tigrinum	2 (12.5)	0
Amblyomma maculatum	3 (18.7)	2 (12.5)
Anocenter nitens	1 (6.3)	1 (6.2)
Boophilus sp.	5 (31.3)	0
Ixodes boliviensis	3 (18.7)	1 (6.2)
Ixodes pararicinus	2 (12.5)	0
Flea species[b]		
Adoratopsilla intermedia	2 (3.4)	0
Ctenocephalides felis	33 (55.9)	2 (3.4)
Ctenocephalides canis	6 (10.2)	0
Pulex irritan	11 (18.6)	0
Neotyphloceras crassispina	2 (3.4)	0
Xenopsylla cheopis	5 (8.5)	0

[a] The percentage based on the total number of either ticks, or fleas.
[b] Fleas of the same species were placed into sorted pools, based on individual hosts.

PCR and phylogentic analysis of a potentially novel SFG agent from ticks. DNA from a total of 16 ticks was screened by the *htrA* PCR. Four ticks were positive for SFG DNA. Of these, two were *Amblyomma maculatum* (tick 047, from a dog, and tick 124, from a horse), one was *Anocenter nitens* (tick 127, from a horse), and the last one was *Ixodes boliviensis* (tick 163, from a horse). Sequence data for the tick 127 *htrA* amplicon

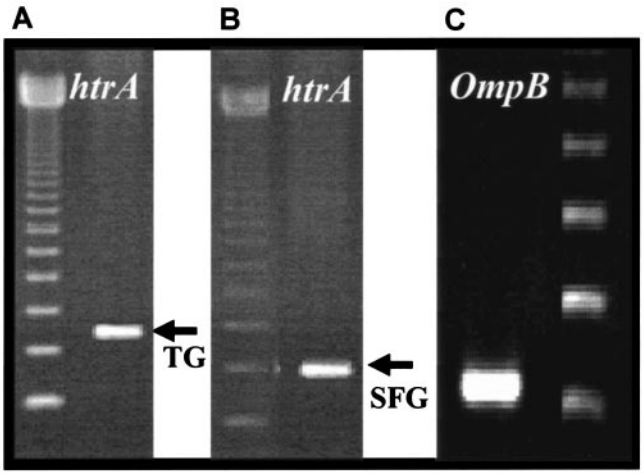

FIG. 1. Representative example of *htrA* gene PCR denoting a positive sample from FL105. Evident are both a TG *Rickettsia*-specific (A) and SFG *Rickettsia*-specific band (B). The 129-bp product of the *ompB* gene amplified from DNA from FL105 with *R. felis*-specific primers is also shown (C). A 123-bp ladder (Gibco Life Sciences, Gaithersburg, Md.) was used as a guide for molecular sizes in all three gel slices.

indicated that this agent was an SFG rickettsia (99.7% homology to the *htrA* sequences of *R. conorii*, *R. rickettsii*, *R. sibirica*, and *R. peacockki*). No additional data were obtained from this sample.

Samples tick 124 and tick 163 yielded positive PCR products for *ompA* (Fig. 2). These samples, as well as tick 047, were also positive by PCR for the citrate synthase gene, *gltA*. Sequencing of the 540-bp *ompA* and the 381-bp *gltA* fragments indicated that they were most closely aligned with the gene sequences of *R. massiliae* (6) and with those of *Rickettsia* sp. strain RPA4 and *Rickettsia* sp. strain DNS14 (isolates from Siberia [44]). To more closely align the experimental sequences with those of known species, a strategy was developed to sequence 2,484 bp of the *ompB* genome with broad-range primers. By this technique, 2,484-bp fragments from tick 124 and tick 163 samples were amplified and sequenced, and then these sequences were compared to those of known *Rickettsia* sp. members (Fig. 3).

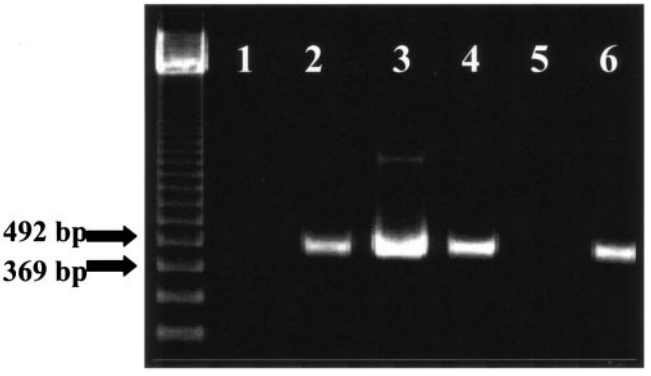

FIG. 2. PCR of the 540-bp *ompA* gene from tick samples. Lane 1, negative extraction control; lanes 2 and 3, positive controls (*R. akari* and *R. rickettsii*, respectively); lanes 4 to 6, ticks 124, 127, and 163, respectively. Samples were electrophoresed in 2% agarose gels.

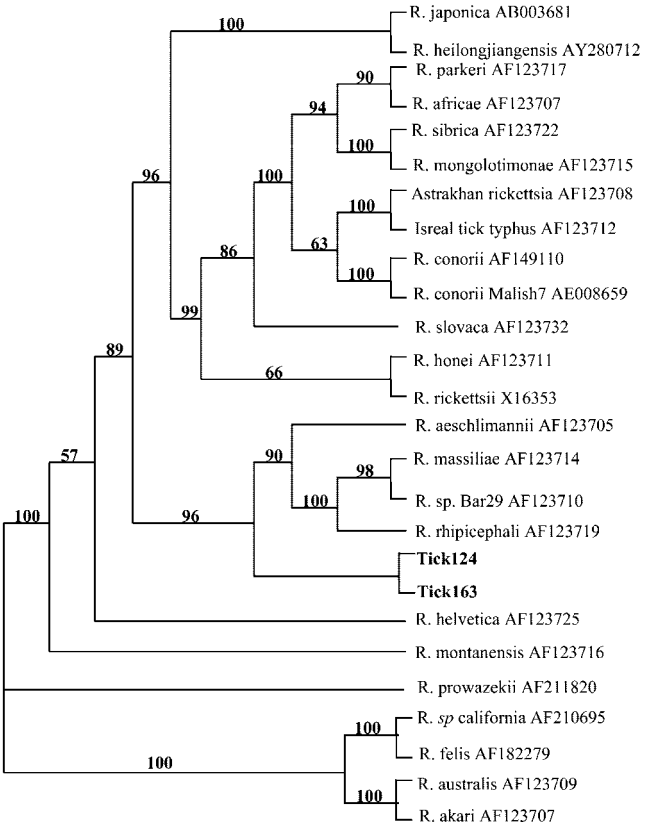

FIG. 3. Dendrogram of the sequence alignment of rickettsia species based on the nucleotide sequences of a 2,487-bp segment of the *ompB* gene. The analysis, including maximum parsimony and neighbor joining, was performed with the PAUP program. Bootstrap values are indicated at the nodes.

With the criterion set to distance, a neighbor-joining search on 100 bootstrap replicates provided node values that bracketed the northern Peruvian tick samples in a phylogenetic tree with described SFG members. Of note, while the sequences from the experimental tick samples were 100% similar, they differed by 3% from the most closely aligned sequences representing the *R. massiliae* group (Table 3).

DISCUSSION

Pathogenic rickettsiae are transmitted to vertebrate hosts by arthropod vectors such as fleas, ticks, lice, and mites. Many of these diseases have resulted in marked morbidity, with occasional fatalities. In the last 10 years, the number of human rickettsioses has dramatically increased, with 15 species recognized throughout the world (25, 31, 37). The incidence of rickettsial pathogens has been underdocumented in Peru, and the species remain undercharacterized. Here we report evidence of SFG rickettsiae among ectoparasites collected during an investigation to determine the zoonotic factors possibly related to an increase in the rate of febrile illnesses in the northwestern region of the country (Table 4).

Toward this end, we used gene sequence-based criteria for the taxonomic positioning of the samples obtained to characterize suspected rickettsiae. Initially, all samples were tested

TABLE 3. Taxonomy comparison of 2,484-bp *ompB* fragment from tick 163

Rickettsia species	GenBank accession no.	No. of identical nucleotides/ total no. tested	% Identity
R. aeschlimannii	AF123705	2,410/2,479	97
R. rhipicephali	AF123719	2,406/2,479	97
Rickettsia sp. strain Bar 29	AF123710	2,402/2,482	96
R. massiliae	AF123714	2,397/2,479	96
R. slovaca	AF123723	2,394/2,479	96
R. conorii Malish	AE008659	2,393/2,479	96
R. conorii	AF149110	2,391/2,479	96
R. parkeri	AF123717	2,390/2,479	96
R. sibirica	AF123722	2,387/2,479	96
R. africae	AF123706	2,387/2,479	96
R. mongolotimonae	AF123715	2,386/2,479	96
R. honei	AF123711	2,385/2,478	96
R. honei strain Thai tick typhus	AF123724	2,383/2,478	96
Isreali tick typhus rickettsia	AF123712	2,379/2,477	96
R. japonica	AB003681	1,809/1,879	96
R. heilongjiangensis	AY280712	2,376/2,478	95
Astrakhan rickettsia	AF123708	2,379/2,479	95
R. rickettsii	X16353	2,377/2,479	95

for expression of the genus-specific gene *htrA* and were then segregated into either the TG or the SFG. Samples were also screened for expression of the citrate synthase gene (*gltA*). The nucleotide sequence of the *gltA* gene serves as a better discriminator of closely related rickettsiae than the 16S rRNA gene (42). To better characterize the rickettsial agents in our samples, *ompA* and *ompB* PCRs were conducted. These genes encode a highly antigenic, large-molecular-mass membrane protein specific for SFG members and have proven useful in delineating relationships (40, 41). Ultimately, phylogenetic relationships among SFG members and our unique sequences were determined by comparison of a large 2,484-bp *ompB* fragment.

The prevalences obtained by these tests demonstrated that 3.3% of flea pools (2 of 59) and 25% of ticks (4 of 16) were positive for SFG agents. Following the *htrA* PCR, no sample yielded solely a TG-specific band, although such a band was evident from two flea samples in combination with an SFG-specific band. The *R. typhi*-specific qPCR assay was negative with this sample, providing evidence that the TG-specific band did not result from a dual *R. felis-R. typhi* infection. The sequence generated after both *htrA* and *ompA* partial gene amplification demonstrated 100% homology to the *R. felis* sequence, which is in a clade closely aligned with but distinct from that for *R. australis* and *R. akari*. These samples were disparate from the flea-borne rickettsiae, *R. typhi*, and the louse-borne agent, *R. prowazekii*, implying genetic distance.

R. felis is an emerging pathogen that was first isolated in a commercial colony of cat fleas (1). Upon isolation, *R. felis* was designated a new species (22) and on the basis of molecular data was placed in the SFG rather than the TG (9). *R. felis* is transmitted by fleas horizontally and vertically (4). It was recognized as a pathogenic rickettsia after identification in samples from the Americas (35). Infected fleas, particularly *C. felis*, were found to be the likely cause of outbreaks in humans in Mexico (53) and Brazil (29). These examples provided evidence that *R. felis* exists in Central and South America. Our samples were taken from *C. felis*, a parasite commonly found on both canines and felines in Peru. We did not find evidence of *R. typhi* among *Xenopsylla cheopis* fleas (3) removed from rats (*Rattus norvegicus*) trapped during our investigation, nor did we identify *R. prowazekii*, the etiologic agent of epidemic typhus (data not shown). However, we did note the presence of nucleic acid extracted from the blood of rodents collected in

TABLE 4. Summary for samples found to be positive for SFG

Sample	Site	Sample description	Host	Gene(s) sequenced	Closest match (%)
FL103	Sapillica	*C. felis*	Dog	*htrA*	*R. felis* (100)
FL105	Sapillica	*C. felis*	Dog	*htrA* *ompA*	*R. felis* (100) *R. felis* (100)
Tick 047	Coletas	*A. maculatum*	Dog	*htrA* *gltA*	SFG (99.7)[a] *R. heilongjiangensis* (98) *Rickettsia* sp. strain RpA4 (98)
Tick 124	Coletas	*A. maculatum*	Horse	*htrA* *ompA* *gltA* *ompB* (2,484 bp)	SFG (98.4) *R. heilongjiangensis* (98) *R. heilongjiangensis* (98) *R. aeschlimannii* (97) *R. rhipicephali* (97)
Tick 127	Coletas	*A. nitens*	Horse	*htrA*	SFG (98.1)
Tick 163	Naranjo	*I. boliviensis*	Horse	*htrA* *ompA* *gltA* *ompB* (2,484-bp)	SFG (99.7) *Rickettsia* sp. strain RpA4 (97.5) *Rickettsia* sp. strain RpA4 (98) *R. aeschlimannii* (97) *R. rhipicephali* (97)

[a] Denotation of SFG represents similar sequence homology to the sequences of four or more SFG members.

Sapillica that was similar to that of *R. felis* (data not shown). *C. felis* is known to feed on mice and rodents and may represent an important reservoir for *R. felis* in northern Peru.

Similar molecular analyses were conducted with 16 ticks removed from domestic animals and animals trapped in the wild in the area of the fever outbreak. Following *htrA* PCR, positive reactions were found for 4 of 16 nucleic acid preparations from ticks, and sequence data obtained from the *ompA* and *gltA* amplicons supported the inclusion of the isolates from these samples in the SFG. To better align our sequences with those established previously, we compared the *ompB* amplicons generated from two ticks (tick 124 and tick 163) with those of other SFG species. The sequences from the Peruvian ticks aligned with sequences from strains just outside a nodule containing the *R. massiliae* group, including *R. rhipicephali* (97%; GenBank accession number AF123719), an isolate from the Astrakhan region of the former Soviet Union (44), *R. aeschlimannii* (97%; GenBank accession number AF123705) from southern Croatia (5, 32), *Rickettsia* sp. strain Bar 29 (GenBank accession number AF123710), and *R. massiliae* (GenBank accession number AF1213714) (6) from the south of France. The tick *R. aeschlimannii* isolate was identified in a patient returning from Morocco. *R. aeschlimannii* is pathogenic for humans; and infection with this rickettsia results in an infection characterized by an eschar, fever, and a generalized maculopapular skin rash (34). *R. rhipicephali* has not been shown to infect humans. Our samples differed from those of the aforementioned SFG members by 3% or more (Table 3). These results infer considerable divergence as well as geographic disparity from the most closely linked matches.

The hard ticks *A. maculatum* and *I. boliviensis* represent species that are common in the Peruvian Andes. Moreover, these are typical vector genera of rickettsial agents and have been shown to transmit disease in regions as far removed as the Pacific coast of the United States (23), Australia (45), and the former Yugoslavian republics (17). Recently, *A. maculatum* has been shown to be naturally infected with *R. parkeri*, a human pathogen in the southern United States (30).

A high serologic prevalence of the bacterium *Leptospira* and SFG rickettsia in the area of the outbreak was determined previously (7), and the SFG rickettsiae from samples from four febrile humans were identified by molecular analyses (7). We have no clear evidence that the SFG rickettsial agent identified by molecular analysis in the four serum samples from febrile humans from northern Peru is the same agent in our tick samples. The patients seen during our study experienced a mild disease typified by fever and headache, often malaise, and less frequently, a stiff neck and joint pain (7). No eschar was reported, and unlike previously reported cases of "spotless" rickettsiosis (for instance, following *R. slovaca* infection [36]), all our patients exhibited fever. The symptoms resolved upon treatment with doxycycline for 1 week.

We report for the first time the identification of *R. felis* from *C. felis* flea samples in Peru. We were also able to amplify conserved rickettsial genes from both a single *A. maculatum* tick and a single *I. bolivensis* tick isolated in northwestern Peru. We do not know whether the SFG agent identified in the tick samples is homologous to that seen in the human samples or, for that matter, whether it can even be transmitted to humans. As was the case for the flea samples, analysis of blood samples taken from rodents trapped during the investigation showed that they were infected with the pathogen *R. felis* (P. J. Blair, unpublished data). However, partial sequencing of the *htrA* gene from human samples suggested infection with another SFG species (P. J. Blair, unpublished). Certainly, the close proximity of the agrarian Andean societies with domesticated canines, felines, and herd animals elevates the risk of vertical transmission. A clearer epidemiological assessment of rickettsial diseases in northwestern Peru will become apparent following isolation and characterization of the agents from host species. Efforts are continuing toward that end. Finally, on the basis of the sequence and phylogenetic data presented herein, we believe that we have identified a putative novel SFG agent, for which we propose the name "*Candidatus* Rickettsia andeanae" in recognition of the area where it was first detected.

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